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R E S E A R C H A R T I C L E

Formulation and Evaluation of Nano-emulsion gel of Quercetin

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A B S T R A C T

The aim of the present study was to develop Nano emulsion formulation for transdermal delivery of the Quercetin to enhance bio availability of the drug. Pseudoternary phase diagrams were constructed to obtain the Nano emulsion region. Labrafac was chosen as oil phase, PEG400,Tween 80 were used as surfactant and co surfactant respectively. EA1 to EA6 different formulations of Quercetin loaded Nano emulsions were prepared successfully by phase titration method and was characterized for particle size, zeta potential, thermodynamic stability study and rheological study.Further, Nano emulsion was incorporated into 1% Carbopol to get a gel for improving convenience in superficial application of drug. EA1 to EA6 different formulations of nanoemulsion gel were prepared and formulations were evaluated for rheological study, Extrudability, swelling index,drug content and invitro drug release studies. Finally it was found that Nano Emulsion EA-6 formulation showed cumulative release as 94%, which shows that Nano emulsion gel has improved absorption and bioavailability.

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C O N T E N T S

1. Introduction

Nano-emulsions consist of fine oil-in-water dispersions, having droplets covering the size range of 10 to less than 1000 nm1. Nanoemulsions, usually spherical, are a group of dispersed particles used for pharmaceuticals biomedical aids and vehicles that shows great promise for

the future of cosmetics, diagnostics, drug therapies and biotechnologies[2].

Drug Profile:

Quercetin isa plant flavanol from the flavonoid group of polyphenols, is found in many fruits, vegetables, leaves and grains. Red onions and kale are common foods containing appreciable content of Quercetin[3].Buck wheat tea has large amount of Quercetin. People use this drug as a medicine.It has a bitter flavour and is used as an ingredient in dietary supplements, beverages and foods.It is a yellow crystalline pigment occurring usually in the form of glycosides. Quercetin is most commonly taken by mouth to treat conditions of the heart and blood vessels and prevent cancer. It is also used for arthritis, bladder infections and diabetes[4].

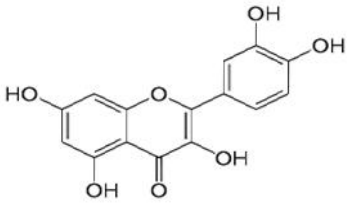

Fig 1: Structure of Quercetin

2. Materials and Methods

Determination of λmax:

100mg of Quercetin was dissolved in 100mL of Sodium Dihydrogen ortho Phosphate buffer(pH 5.5) in 100 mL volumetric flask and filtered. From the above solution pippete out 1mL of the filtrate and dilute to 100mL with the same solvent and determine the λmax using U.V. spectrophotometer.

Estimation of Quercetin:

In present study, the spectrophotometric method was adopted for the estimation of Quercetin using double beam U.V. spectrophotometer.

Standard Calibration:

50 mg of Quercetin was accurately weighed and dissolved in 50 ml of phosphate buffer pH 5.5 to give a stock solution of 1mg/ml (1000µg/ml) concentration. This was served as a blank. From this 10ml of the solution was taken and diluted to 100ml to get a solution of 100 µg/ml and this was served as a standard solution. Into a series of 10 ml volumetric flask, aliquots of standard solution i.e. 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml and 3.0ml were added and the volumes were made up to 10 ml using phosphate buffer. The absorbance of these solutions was measured against the reagent blank at 260 nm using UV- spectrophotometer. A standard curve was plotted with concentration on X-axis and absorbance on Y-axis.

Pre-formulation Studies:

Screening of Oils, Surfactants and Cosurfactant by Solubility Studies of Drug:

Solubility of Quercetin in various oils, surfactants and co surfactant was determined by adding excess amount of 50°C in a drug (approx 500 mg) in screw-capped vials containing 2 mL of vehicle.The mixture was heated at water-bath to facilitate the solubilization using vortex mixer. Mixtures were shaken with shaker at 37°C for 48 h. After reaching equilibrium each vial was centrifuged at 3000rpm for 15min, and excess insoluble methotrexate was discarded by filtration using a membrane filter (0.45 adding excess amount of drug (approx 500 mg) in screw-capped vials containing 2 mL of vehicle. The mixture was heated at 50°C in a water-bath to facilitate the solubilization using vortex mixer. Mixtures were shaken with shaker at 37°C for 48 h. After reaching equilibrium each vial was centrifuged at 3000rpm for 15min, and excess insoluble methotrexate was discarded by filtration using a membrane filter (0.45µm, 13mm, Whatmann USA). The concentration of drug was quantified by measuring the absorbance at 259nm using UV visible spectrophotometer.

Solubility in solvents:

Excess amount of drug was dissolved in methanol, 5.5 pH phosphate buffer, 6.8 pH phosphate buffer and solubility was determined using UV spectrophotometer at 260nm.

Screening of oils, surfactant and co-surfactant:

Excess of drug in 2ml of each of selected oils were taken in 5 ml stopped vials, mixed and kept at 37±1oC in a shaker for 72 hr. After 72 hours sample was removed and centrifuged at 3000 rpm for 15 min. Dilutions of these solutions were prepared in methanol from 2 to 10 ppm. The absorbance of each standard solution was determining spectro photometrically at 260nm.

Pseudo ternary phase diagram:

On the basis of solubility study of drug, labrafac was selected as the oil phase. Tween 80 and PEG400 were selected as surfactant and co-surfactant as per their emulsification capability for the system. Distilled water was used as an aqueous phase for the construction of phase diagram for the determination of existence zone of nanoemulsion. Pseudo ternary phase diagrams were constructed using aqueous titration method. To construct pseudo ternary phase diagrams the oil phase was mixed with surfactant: co-surfactant and titrated against distilled water in the ratio of 1:1, 1:2, 2:1. The mixture was titrated with distilled water until it turned turbid. The volume of water used was recorded.

Compatibility study

Fourier transforms Infrared spectroscopy (FT-IR):

The IR Spectra of Quercetin and excipients were recorded by Shimadzu 8400 FT-IR spectrophotometer. Sample was prepared by KBR disc method and examined in the transmission mode. Spectrum was measured over frequency range of 4000-400 cm^{-1}. The peaks obtained in the spectra were then compared with the corresponding functional groups in structure of Quercetin.

Preparation of Nano emulsion of Quercetin:

In this study formulations were prepared by Phase titration method. Appropriate amount of surfactant and co-surfactant were mixed and then added oily part, mixed the formulation until completely dispersion occurs at room temperature. Then drug was added and the final mixture was mixed by overtaxing until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 hrs, and examined for signs of turbidity or phase separation. The formulation was designed using labrafac as oil phase and Tween 80/PEG-400 as surfactant/co-surfactant.

Characterisation of Nano emulsion

Physical appearance:
The prepared formulations were inspected visually for their colour and appearance.

Particle size Measurement:
Particle size of nanoemulsion was measured by Scattering light intensity at scattering angle 90 C temperature

Zeta potential measurement:
Zeta potential of nanoemulsion was measured at temperature 25 C and viscosity of dispersion medium 0.895at conductivity 0.098ms/cm and electro voltage 3.9V.

Thermodynamic stability study:
Thermodynamic stability of the Nano emulsion system was determined by performing following tests.

Heating Cooling Cycle:
Nanoemulsion formulations were subjected to six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature not less than 48h. Stable formulations were then subjected to centrifugation test.

Centrifugation:
Nanoemulsion formulations were centrifuged at 3500 rpm for 30 min and those formulations which did not show any phase separation were taken for the freeze-thaw stress test.

Freeze-Thaw Cycle:
In this the formulation was subjected to three freeze-thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 h was done for the formulations.

Rheology study of Nano emulsion:
The viscosity of Nano emulsions of different formulations was measured at 10 rpm for 3 min at 25oC by Brookfield type rotary viscometer.

Preparation of Gel:
1% Carbopol was selected as the gel matrix to prepare the Nano emulsion based hydrogel formulation. Carbopol was slowly mixed with the nanoemulsion under stirring. AfterCarbopol had swelled, it was kept overnight to obtain the Nano emulsion-based hydrogel.

Characterization of gel

Physicalappearance:
The prepared Nanoemulsion gel formulations were inspected visually for their color, homogeneity, consistency, grittiness and phase separation.

pHDetermination:
pH determination of prepared formulations was done by using digital pH meter. The procedure was carried out by taking Nanoemulsion Gel in 250 ml beaker immersing pH meter into the formulation and readings of pH meter were recorded. Same process was repeated two more times with the same formulation.

Extrudability:
The Extrudability test was carried out using hardness tester. A 5 gm of Nanoemulsion Gel was filled into the aluminum collapsible tubes. The plunged is subjected to hold the tube properly. The 1gm/cm2 applied for the 30 sec. The measured quantity of Nanoemulsion gel extruded from the tube repeat procedure for three times.

Swellingindex:
To determine the swelling index of prepared Nanoemulsion gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1% NaOH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows.

Swelling Index (SW) % = [(Wt – Wo) / Wo] ×100

Where

(SW)%=Equilibrium percent swelling. Wt = Weight of swollen emulsiongel after time t. Wo = Original weight of emulsiongel at zero time.

Drug contentdetermination:
1.33gm of Nanoemulsion gel was taken dissolved using 100ml of methanol and sonicate for the period of 15 min filtered it by whatmann filter paper. Further dilutions were made by using methanol prepared concentration within Beer's range. The absorbance was measured at 272 nm by UV-Visible spectrophotometer and drug content was determined

Viscosity of the gel:
The viscosity of gel of different formulations was measured at 10 rpm for 3 min at 25oC by Brookfield type rotary viscometer with spindle.

In-vitro releasestudies:
The in-vitro drug release studies were carried out using a Franz diffusion cell. The formulation was applied the surface of egg membrane which was placed between donor and receptor compartment of the Franz diffusion cell. Phosphate buffer pH 5.5 was used as a dissolution media. The temperature of the cell was maintained at 37oC by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. Sample (5 ml) was withdrawn at suitable time intervals and dilute up to 10ml with same solvent and replaced with equal amounts of fresh dissolution media. Samples were analyzed spectrophotometrically at 271 nm and the cumulative % drug release was calculated.

3. Results and Discussion

λ max determination – 260 nm

Solubility study: Solubility of Quercetin in different solvents are shown in Table 1. Drug showed more solubility in phosphate buffer of pH 6.8 than in phosphate buffer of pH 5.5 and methanol.

Table 1: Solubility of Quercetin in different solvent

S. No.	Solvent	Solubility mg/ml
1	Methanol	18.4
2	Phosphate buffer of p^H 5.5	24.5
3	Phosphate buffer of p^H 6.8	33.3

Standard Calibration chart of Quercetin against 5.5 pH buffer:

Table 2: Standard Calibration

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.324
3	4	0.412
4	6	0.612
5	8	0.701
6	10	0.902

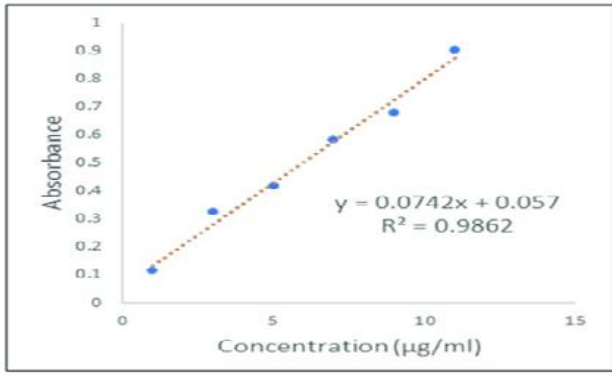

Fig 2: Calibration curve of Quercetin

Pseudo-Ternary Phase Diagram Study:

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations having minimum surfactant concentration, in the least possible time. Ternary phase diagrams were constructed by varying Tween 80: PEG-400 ratios as 1:1, 1:2, and 2:1. The shaded areas of phase diagrams show the nanoemulsion regions, whereas the non-shaded area displays the emulsion region. Thus, the ternary phase system of Tween 80: PEG-400, (1:1, 2:1) that exhibited maximum area for nanoemulsion formation was selected for the optimization of nanoemulsion batches. It was clearly evident that an increase in the concentration of Tween 80 resulted in a decrease in globule size but 1:2 ratio of Tween 80: PEG-400 does not show the long time stability so these are rejected.

Compatibility study:

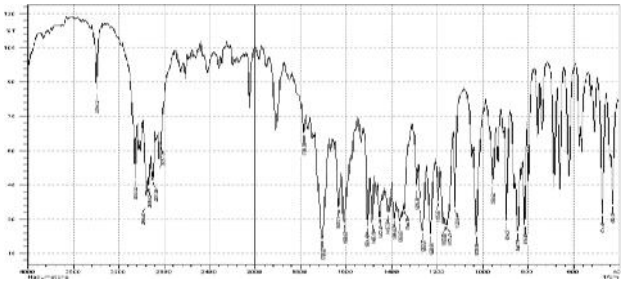

Fig 3: FT IR spectrum of Quercetin

Characterization of Nano emulsion

Physicalappearance: Formulation was examined for appearance which shows transparent formulation. They do not show any turbidity.

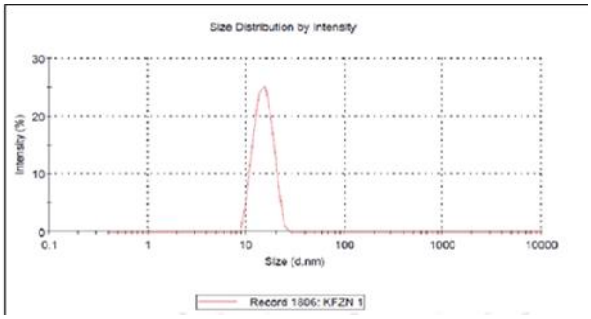

Fig 4: Particle size distribution of nano emulsion

Table 3: Particle Size Distribution

S.No.	Formulation code	Particle size(nm)
1	F-A1	42.32
2	F-A2	53.12
3	F-A3	48.34
4	F-A4	38.19
5	F-A5	42.32
6	F-A6	41.65

The result showed that the particle size of formed nanoemulsion was in the required range as peak was found to be at 50, therefore, a transparent nanoemulsion formulated successfully.

Zeta potential measurement:

Table 4: Zeta Potential Measurement

Peak no	Zeta potential	Electrophoretic mobility
1	-0.1Mv	-0.000001 cm^2/Vs

Zeta potential (mean) = -0.1mV, Electrophoretic mobility (mean) = -0.000001 CM2/VS.

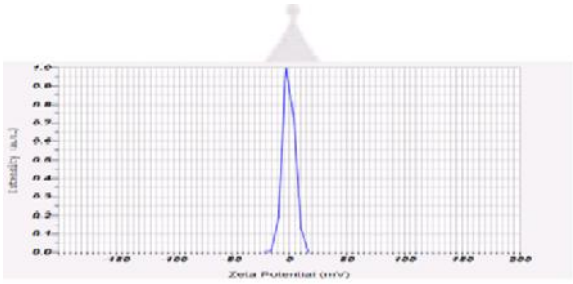

Fig 5: Zeta potential Graph of Nano emulsion

Viscosity of Nanoemulsion:

From the above study, it was concluded that formulation F-A2, F-A4, F-A5 and F-A6 are thermodynamically stable and formulation F-A1and F-A3 are thermodynamically unstable.

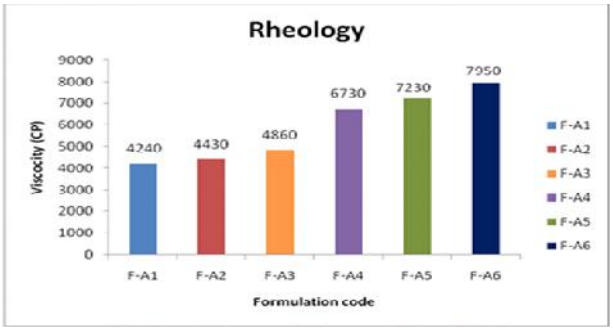

Fig 6: Viscosity study of Nano emulsion

The formulation F-A6 shows the high viscosity and formulation F-A1 shows low viscosity. The viscosity of nanoemulsion depends on the nature and concentration of emulsifying agents.

pH determination:

The pH of gel in between 5.5 to 6 which lies in between normal pH range of skin which does not produce any skin irritation.

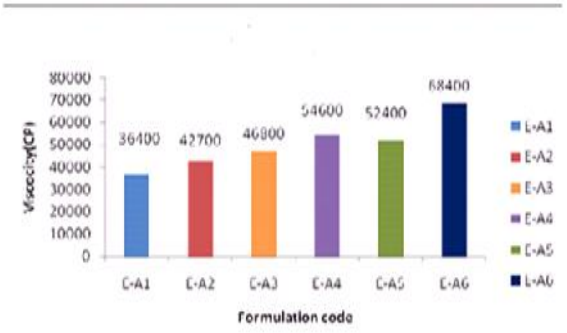

Fig 7: Viscosity study of Nano emulsion gel

The formulation E-A6 showed high viscosity and formulation E-A1 showed low viscosity. The viscosity of Nano emulsion gel depends upon the concentration and nature of gelling and emulsifying agents.

In-vitro release studies:

The in-vitro release of Quercetin Nano emulsion gel was varied in amount according to concentration of emulsifying agents used on formulations. The release of drug was in following ascending order E-A1 < E-A3 < E-A4 < E-A2 < E- A6 < E-A5. Where amount of % release 44.785% < 68.829% < 75.95% < 78.03% <81.37%< 87.63% < 94.937%. From the study it was concluded that E-A5 nanoemulsion gel showed better drug release within 300 minutes.

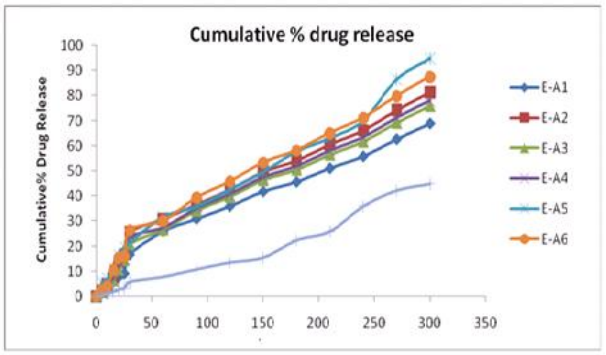

Fig 8: Cumulative % drug release of nanoemulsion gel

4. Conclusion

Preformulation studies were carried out using the spectrophotometric method of estimation of Quercetin and to investigate any possible drug polymer interaction FTIR studies were revealed that there was no drug polymer interaction in the formulation Total of six formulations were prepared all the formulations of QuercetinNano emulsions were characterized by physicochemical evaluation.The QuercetinNano emulsion gel showed viscosity in the range. Amount of Quercetin released from formulations in 5hours ranges from 68.6% to 94.4%.The best drug release was shown by formulation E-A5.This concludes that Quercetin Nano emulsion gel can be expected to show better bioavailability than the conventional system. Nanoemulsion gel shows potential drug delivery system with good stability and release profile. All other formulations were also equally good in their physicochemical characteristics.

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